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Pathway mapping reveals antiretroviral treatments' targeted cell cycle regulation in lung cancer

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ABSTRACT

The human cell cycle is a tightly regulated process with checkpoints in place to ensure genomic integrity. Cyclin/cyclin dependent kinases (CDKs) complexes drive the progression of the cell cycle while CDK-inhibitors (CDKIs) halt the cell cycle. Deregulation of the cell cycle is a hallmark of lung cancer. TP53 and FOXO transcription factors share similar mechanisms in the regulation of the cell cycle. Lung carcinogenesis in the antiretroviral (ARV) era remains to be understood. This study aimed at mapping the biological pathways related to the human cell cycle that are influenced by the ARVs (Efavirenz-EFV and Lopinavir/ritonavir-LPV/r) treatment in A549 and MRC-5 lung cells. For this purpose, Reactome database was used to map these pathways. In addition, the Database for Annotation, Visualisation and Integrated Discovery (DAVID) was used for functional enrichment analysis in a set of genes and to visualise differentially expressed genes within a particular KEGG pathway, and also to perform Gene Ontology. Furthermore, the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used to determine the protein-protein interaction of the differentially expressed gene (DEG) targets. Reactome analysis revealed a decrease in DNA replication and an increase in response to external stimuli and DNA repair genes in both normal and adenocarcinoma lung cancer models. A further increase in apoptotic genes is observed in the cancer cells in response to ARV treatment. Interestingly, the FOXO pathway was also shown to be upregulated in the test (ARV) groups. KEGG pathway shows a reduction in cyclin/CDK activity in ARV treated models. STRING analysis illustrates a direct and strong interaction between DNA damage and response (DDR) genes upregulated by ARV exposure. Analysis from all three databases suggests the cytotoxic and anti-proliferative effects of EFV and LPV/r on lung cancer.

1. Introduction

The human cell cycle is a highly regulated and precise process [1,2]. The high fidelity of the cell cycle is achieved by its checkpoints. These surveillance mechanisms monitor the cell cycle progression by ensuring an interdependency of synthesis (S)-phase and mitosis (M), the integrity of the genome and the fidelity of chromosome segregation [3,4]. Genomic DNA replication followed by the chromosomal segregation into daughter cells make up the cell cycle. The S-phase replicates DNA and M-phase separates chromosomes. The periods between the two phases are known as the gap phases (G1 and G2) [2]. Cells can temporarily exit the cell cycle and enter a quiescent phase known as G0. Alternatively, cells can terminally differentiate into cells that will cease to divide but undergo morphological development to carry out a variety of specialized functions of individual tissues [2,5].

The cell cycle is positively regulated, thereby driving its progression and negatively regulated, halting the events of this process [6,7]. The cyclin/CDK complexes drive the cell cycle. On the other hand, the CDK-inhibitors (CDKIs) inhibit the activity of CDKs when the DNA damage checkpoints are activated. As a result, the phosphorylated CDKs remain inactive and are unable to bind cyclins [8,9]. Failure to properly repair the damaged DNA through DNA damage response pathways (DDR) or direct the cell to programmed cell death (PCD)/apoptosis may lead to pathologic conditions such as cancer (Guarino et al., 2020; [10–13].). In addition, TP53 and FOXO transcription factors independently induce the transcription of genes involved in cell cycle arrest

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Table 1
The layout of the human cell cycle gene array with gene names (abbreviated) and GenBank accession numbers.

	1	2	3	4	5	6	7	8	9	10	11	12
A	ABL1	ANAPC2	ATM	ATR	AURKA	AURKB	BCCIP	BCL2	BIRC5	BRCA1	BRCA2	CASP3
	NM_005157	NM_013366	NM_000051	NM_001184	NM_003600	NM_004217	NM_016567	NM_000633	NM_001168	NM_007294	NM_000059	NM_004346
B	CCNA2	CCNB1	CCNB2	CCNC	CCND1	CCND2	CCND3	CCNE1	CCNF	CCNG1	CCNG2	CCNH
	NM_001237	NM_031966	NM_004701	NM_005190	NM_053056	NM_001759	NM_001760	NM_001238	NM_001761	NM_004060	NM_004354	NM_001239
C	CCNT1	CDC16	CDC20	CDC25A	CDC25C	CDC34	CDC6	CDK1	CDK2	CDK4	CDK5R1	CDK5RAP1
	NM_001240	NM_003903	NM_001255	NM_001789	NM_001790	NM_004359	NM_001254	NM_001786	NM_001798	NM_000075	NM_003885	NM_016408
D	CDK6	CDK7	CDK8	CDKN1A	CDKN1B	CDKN2A	CDKN2B	CDKN3	CHEK1	CHEK2	CKS1B	CKS2
	NM_001259	NM_001799	NM_001260	NM_000389	NM_004064	NM_000077	NM_004936	NM_005192	NM_001274	NM_007194	NM_001826	NM_001827
E	CUL1	CUL2	CUL3	E2F1	E2F4	GADD45A	GTSE1	HUS1	KNTC1	KPNA2	MAD2L1	MAD2L2
	NM_003592	NM_003591	NM_003590	NM_005225	NM_001950	NM_001924	NM_016426	NM_004507	NM_014708	NM_002266	NM_002358	NM_006341
F	MCM2	MCM3	MCM4	MCM5	MDM2	MKI67	MNAT1	MRE11A	NBN	RAD1	RAD17	RAD51
	NM_004526	NM_002388	NM_005914	NM_006739	NM_002392	NM_002417	NM_002431	NM_005590	NM_002485	NM_002853	NM_002873	NM_002875
G	RAD9A	RB1	RBBP8	RBL1	RBL2	SERTAD1	SKP2	STMN1	TFDP1	TFDP2	TP53	WEE1
	NM_004584	NM_000321	NM_002894	NM_002895	NM_005611	NM_013376	NM_005983	NM_005563	NM_007111	NM_006286	NM_000546	NM_003390
H*	ACTB	B2M	GAPDH	HPRT1	RPLP0	HGDC	RTC SA_00104	RTC SA_00104	RTC SA_00104	PPC SA_00103	PPC SA_00103	PPC SA_00103
	NM_001101	NM_004048	NM_002046	NM_000194	NM_001002	SA_00105						

[14]. This cell cycle arrest coordinated by the checkpoints provides cells an opportunity to repair the damage prior to cell division. In so doing, the transmission of genetic errors to daughter cells is prevented [10,14]. Furthermore, cell cycle arrest allows cells an opportunity to recover from the damaged DNA and survive, thus preventing premature and unnecessary cell death, which can also be pathologic as genomic DNA is constantly facing both intrinsic and extrinsic damaging effects [5,15]. Failure to properly regulate the cell cycle is a hallmark of cancer, including lung cancer [16]. Lung cancer is a leading cause of morbidity in both the HIV negative and HIV positive populations [17]. Furthermore, non-small cell lung carcinoma (NSCLC) accounts for >80% of the cases, adenocarcinoma is the most common type [18]. Since the advent of combination antiretroviral therapy (cART) also known as the highly active antiretroviral therapy (HAART), the quality of life for HIV positive people has improved. On the contrary, HIV/AIDS co-morbidities such as lung cancer have been reported to be on the rise [19,20]. In the present HAART era, very little is known about the presentation of lung cancer in HIV-infected individuals [17]. This highlights the importance of research on lung cancer in HIV and treatment. The potential regulatory role of ARVs on lung cancer and cell cycle has not yet been explored. Thus, this study aimed at determining the role of two ARV drugs (efavirenz-EFV and lopinavir/ritonavir-LPV/r) which form part of HAART, in the regulation of the human cell cycle. For this purpose, *in silico* bioinformatics analysis was employed to map the biological pathways, perform Gene Ontology (GO) analysis and determine gene/gene or protein/protein interactions in response to ARV treatment. EFV and LPV/r may activate/repress pathways related to the cell cycle, altering the balance between cell death and cell proliferation.

2. Materials and methods

Primary lung fibroblasts (MRC-5) and adenocarcinoma lung cells (A549) were treated with clinical plasma doses of 13 μM EFV and 32 μM LPV/r for 48hrs as previously described in Marima et al., [21]. Briefly, the lung MRC-5 (ATCC CCL171) and A549, (ATCC CCL185) were obtained from the American Type Culture Collection (ATCC). MRC-5 and A549 cells were grown in Dulbecco's Modified Eagle Medium (DMEM, Life Technologies, ThermoFischer) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (MilliporeSigma) and 1% penicillin and streptomycin (GIBCO, ThermoFischer). Cells were cultured in 25 cm^2 cell culture flasks (Corning) and were kept in a CO_2 incubator at 37 °C in a humidified atmosphere with 5% CO_2 in air. For experimental purposes, cells cultured to an exponential growth phase (at approximately 70% confluency) were used. Cells were then serum-starved for 24hrs to synchronise the cell cycle. The following day, the cells were pharmacologically treated with either 13 μM EFV or with 32 μM LPV/r for 48hrs. Control cells were exposed to growth medium and vehicle only (methanol 0.1% v/v).

The differential gene expression gene (DEG) patterns were calculated using Qiagen web-portal (geneglobe.qiagen.com) and expressed as fold changes. The ±2 and $p < 0.05$ were considered significant for the DEGs. Three bioinformatics tools, Reactome database v72, DAVID database v6.8 and STRING database v11.0 were used to map the biological pathways, determine functional enrichment and protein/protein interaction in response to ARV treatment. The human cell cycle PCR array (96-well format; PAHS-020Z, Qiagen, Table 1) was used. Rows A to G (1–84) genes are cell cycle related, while the H row exclusively refers to controls including trademark controls, for quality assurance of this assay.

2.1. Reactome database v72

Reactome database https://reactome.org/version 72 was used to map the biological pathways in the test (ARV treated) and control (non-ARV treated) groups. The analysis tool was selected for data analysis. A list of GenBank accession numbers of differentially expressed genes of

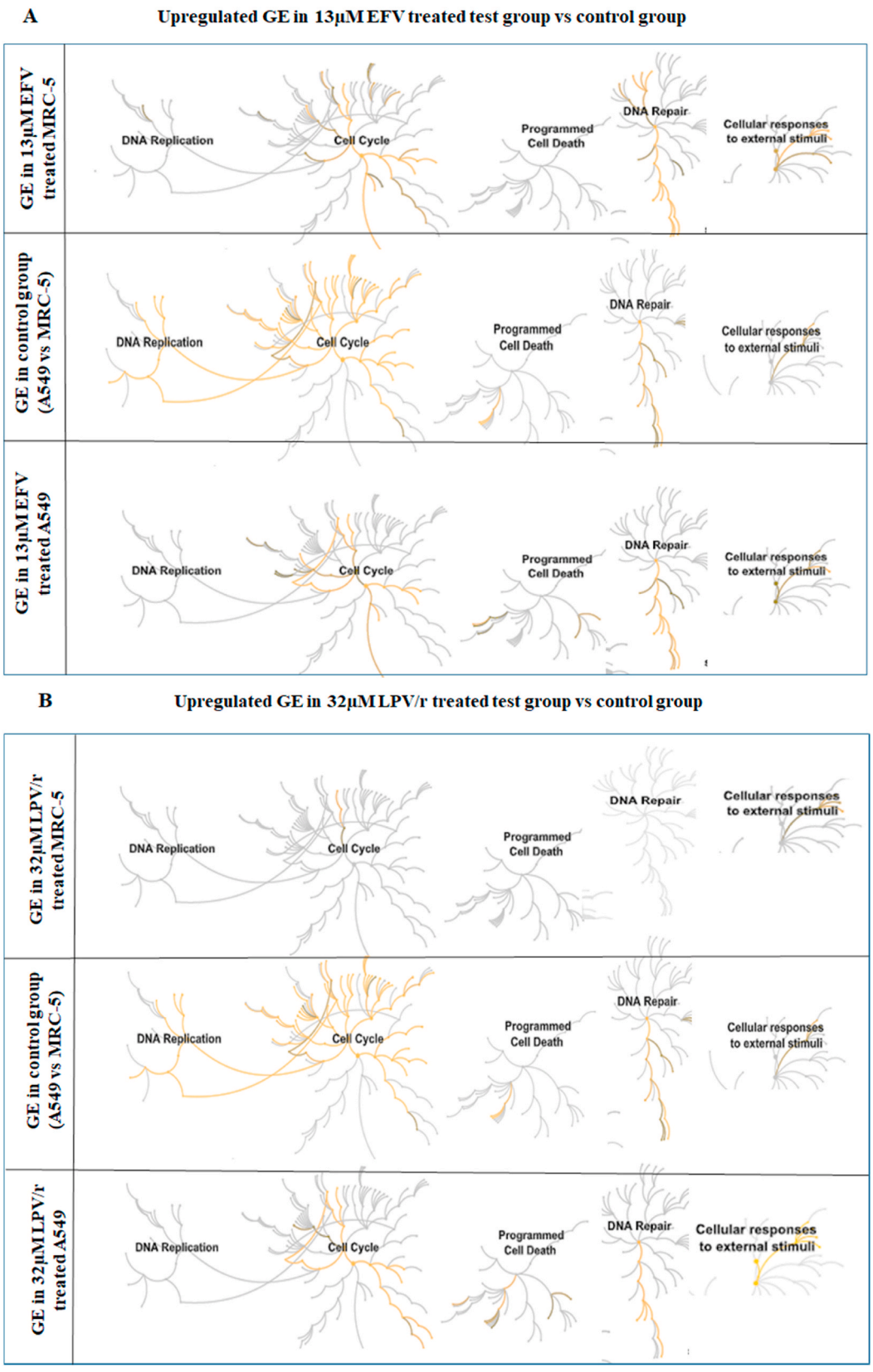

Fig. 1. The Reactome map illustration of biological/molecular pathways influenced by the identified gene targets in response to ARV drug (**A**-EFV and **B**-LPV/r) treatment. Change in gene expression (GE) patterns of the targets was compared between ARV treated and control groups. Change in GE patterns is represented between four pathways related to the cell cycle: Cellular response to external stimuli, DNA repair, programmed cell death (PCD)/apoptosis and DNA replication. In response to ARV drug treatment, the DNA repair genes are up-regulated in both normal and cancerous cells, extending to the activation of apoptosis genes in the A549 cells, while a decline in DNA replication is observed in ARV-treated groups.

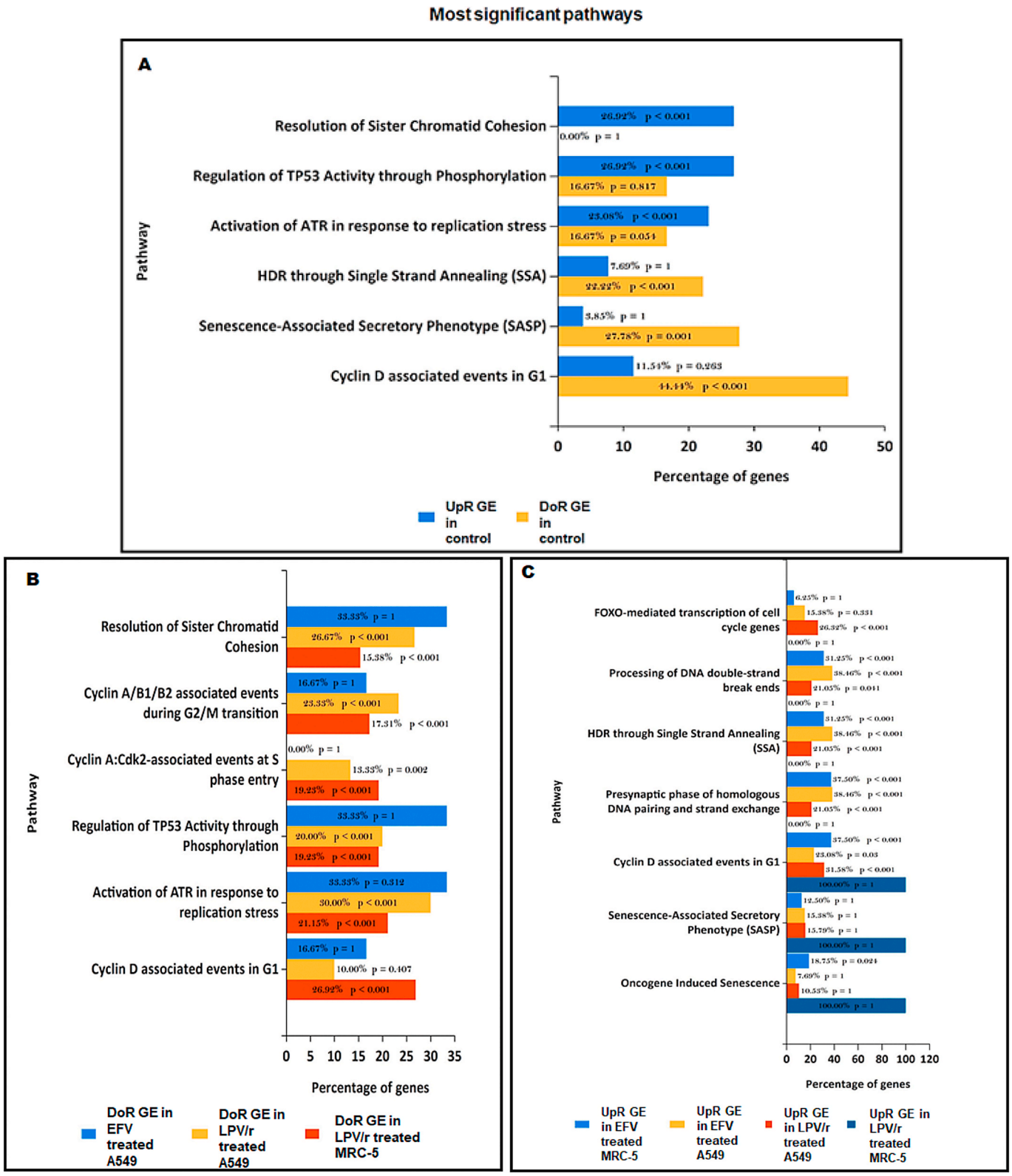

Fig. 2. DEGs in the most significant pathways in control and test groups. This is a representation of the up and downregulated in control (**A**), and downregulated genes (**B**) and genes and upregulated genes (**C**) in test groups, with $p < 0.001$ considered significant. UpR- Upregulated genes; DoR – Down regulated genes.

the test and control groups was uploaded. The human identifiers and IntAct interactors functions were selected to increase the analysis background. The genome wide overview was created by an over-representation analysis. This is a statistical (hypergeometric distribution) test that determines whether certain Reactome pathways are over-represented (enriched) in the submitted data. This test produces a probability score, which is corrected for false discovery rate (FDR) using the Benjamani-Hochberg method, i.e a binomial test is used to calculate the probability shown for each result, and the p-values are corrected for the multiple testing (Benjamini–Hochberg procedure) that arises from

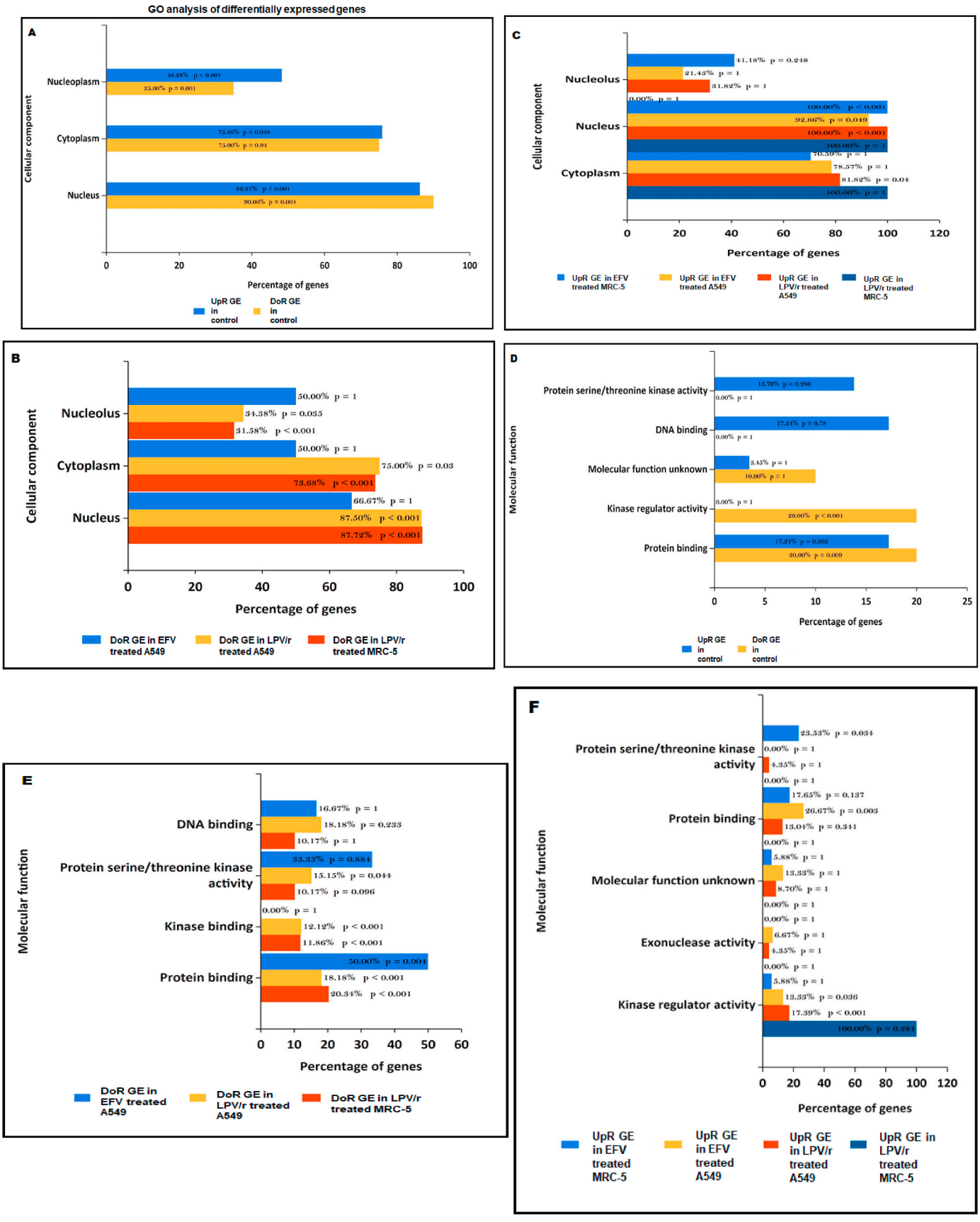

Fig. 3. Gene Ontology (GO) analysis of cell cycle related genes in control and ARV treated groups. GO analysis was done to determine the localization of the significantly DEGs and their molecular function. **A** represents localization of the DEGs in the control group, **B** shows localization of the downregulated while **C** shows for upregulated GE in test groups. **D**, **E** and **F** refer to the molecular function of DEGs in the control group, downregulated GE and upregulated GE in test (ARV) groups. UpR- Upregulated genes; DoR – Down regulated genes.

evaluating the submitted list of identifiers against every pathway.

2.2. DAVID database v6.8

The Database for Annotation, Visualisation and Integrated Discovery (DAVID) version 6.8 (david.ncifcrf.gov) was used to enhance gene functional enrichment. The functional annotation tool was selected for data analysis. The gene list was uploaded selecting the GenBank accession numbers identifier. The Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway was selected for visualisation. Additionally, Gene Ontology (GO) was performed using the DAVID tool.

2.3. STRING database v11.0

Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) database was used https://string-db.org/to determine the protein-protein interaction. A list of GenBank accession numbers of differentially expressed genes from the test and control groups was uploaded on the STRING database, multiple proteins and homo-sapiens functions were selected. The minimum required interaction score was set at the medium confidence-0.4.

The interaction network may be advantageous to represent information other than lists of genes or pathways, as it describes which genes are closely connected within a given pathway. Hence, it has the potential to detect more indirect signals, such as local disturbances within known pathways, as well as within pathways that may not yet have been described [22].

3. Results

3.1. Overview

Results from all three databases show that the input list of genes closely interact, and they are related to the cell cycle focused pathway. A general decrease in expression of DNA synthesizing genes and cyclin/CDK complexes in response to ARVs was observed. Upregulation of kinase regulators which act as cell cycle negative regulators, CDKIs and downstream of FOXO pathway was also seen post ARV treatment. The ARV treatment seems to be positively targeting the kinase regulation (i.e CDKI), which in turn negatively regulates protein binding (cyclin/CDK) interaction. A collective decrease in pathways that promote cell survival and proliferation and activation of anti-cell proliferation pathways is evident in response to ARV treatment.

3.2. Reactome

In this study, Reactome version v72 was used to map and analyse biological and molecular pathways related to the cell cycle pathway, which may be influenced by the differentially expressed gene targets. Within the generated maps, gene expression level corresponds to the yellow colour, and the degree of expression correlates with the colour intensity, Fig. 1. In addition to the genome wide overview (Fig. 1), the most significant pathways with $p < 0.001$ are illustrated, Fig. 2.

3.2.1. Reactome pathway mapping and genome wide analysis

This revealed that EFV and LPV/r drug treatment altered the expression of the profiled cell cycle related genes, affecting other pathways besides the cell cycle. In particular, EFV was shown to activate the cellular response to external stimuli genes in MRC-5 cells, DNA repair genes in both normal and cancerous cells, while upregulated PCD genes and an obvious decline in expression of genes involved in DNA replication in A549 cells was observed compared to the control cells (Fig. 1A). Inhibiting DNA synthesis is an important therapeutic strategy that is widely used to treat a number of hyperproliferative diseases such as cancer [23]. Additionally, CDKIs have great potential as anti-cancer drugs [1]. EFV could be targeting/upregulating CDKIs, thereby regulating or reducing DNA replication, in A549 cells. LPV/r treatment results in the reduced expression of DNA replication in both normal and cancerous cells, while an increase in genes involved in PCD in the cancerous cells was observed (Fig. 1B). In particular, both EFV and LPV/r treatment upregulate apoptosis induced by DNA fragmentation as well as stimulation of cell death response by p21-activated protein kinase 2 (PAK-2p34) in the adenocarcinoma cells. PAK –p34 stimulates cell death response in response to stress signals [24,25], while no evidence seen here of apoptosis induction by these ARVs in normal primary fibroblasts, except for the increase in cellular response to external stimuli in these cells. Next, most significant pathways are represented, Fig. 2.

3.2.2. Most significant pathways as revealed GO analysis

With regards to pathways that are mostly influenced by the ARVs in lung cancer cells, the significantly downregulated pathways (Fig. 2B) particularly in response to LPV/r in both MRC-5 and A549 cells include the resolution of sister chromatid cohesion (this pathway is significantly upregulated in the control group, Fig. 2A). This process involves the separation of sister chromatids [26]. Observably, the main drivers of the cell cycle progression such as cyclin A and B associated events during the G2/M transition, cyclin D associated G1 events as well as cyclin A:CDK 2 associated events at S-phase entry are downregulated (Fig. 2B). Surprisingly, the cyclin D G1 associated events pathway is downregulated in the control group. Interestingly, the most significantly activated pathways in response to ARVs include the processing of dsDNA breaks, homologous DNA repair (HDR) through single strand annealing (SSA), presynaptic phase of homologous DNA pairing and strand exchange. These pathways play a crucial role in the DNA damage and repair pathways. Furthermore, the significant upregulation of FOXO-mediated transcription of cell cycle genes is evident here (Fig. 2C). The FOXO pathway induces the expression of several genes that negatively regulate the proliferation of cells [27,28].

3.3. DAVID

The Database for Annotation, Visualisation and Integrated Discovery (DAVID) v6.8 was used for functional enrichment analysis in a set of genes, to visualise differentially expressed genes within a particular KEGG pathway. Furthermore, this database was used for Gene Ontology (GO) analysis, Fig. 3.

3.3.1. Gene Ontology (GO) analysis

GO was further used to describe the observed patterns from the Reactome database. GO describes gene products with three independent categories: the cellular component, molecular function and biological process [29–31]. A probability level of $p < 0.001$ was considered significant. Fig. 3 refers to GO analysis.

The nucleolus as site of ribosome biogenesis plays a key role in cell metabolism. Most of the significantly up-and downregulated genes (DEGs) are in the nucleus in both control and test groups. This indicates that these DEGs are not mainly involved in the ribosomal RNA biogenesis, or protein synthesis but rather in mitotic cell division or cell proliferation (Fig. 3A, B and C). Furthermore, the molecular function analysis of these DEGs in test groups points to the downregulated activity ($p < 0.001$) of protein kinase binding in both normal and cancerous cells (Fig. 3E), and significantly upregulated activity of kinase regulation, Fig. 3F, (this activity is significantly downregulated in the control group, Fig. 3D), which mostly functions at the cell cycle checkpoints (Fig. 3D), thereby halting the progression of the cell cycle. This is particularly observed in LPV/r treatment in both normal and cancerous cells.

3.3.2. KEGG pathway analysis using DAVID tool

Within the KEGG representations, the red stars represent the input target genes/products; the green boxes represent genes related to the

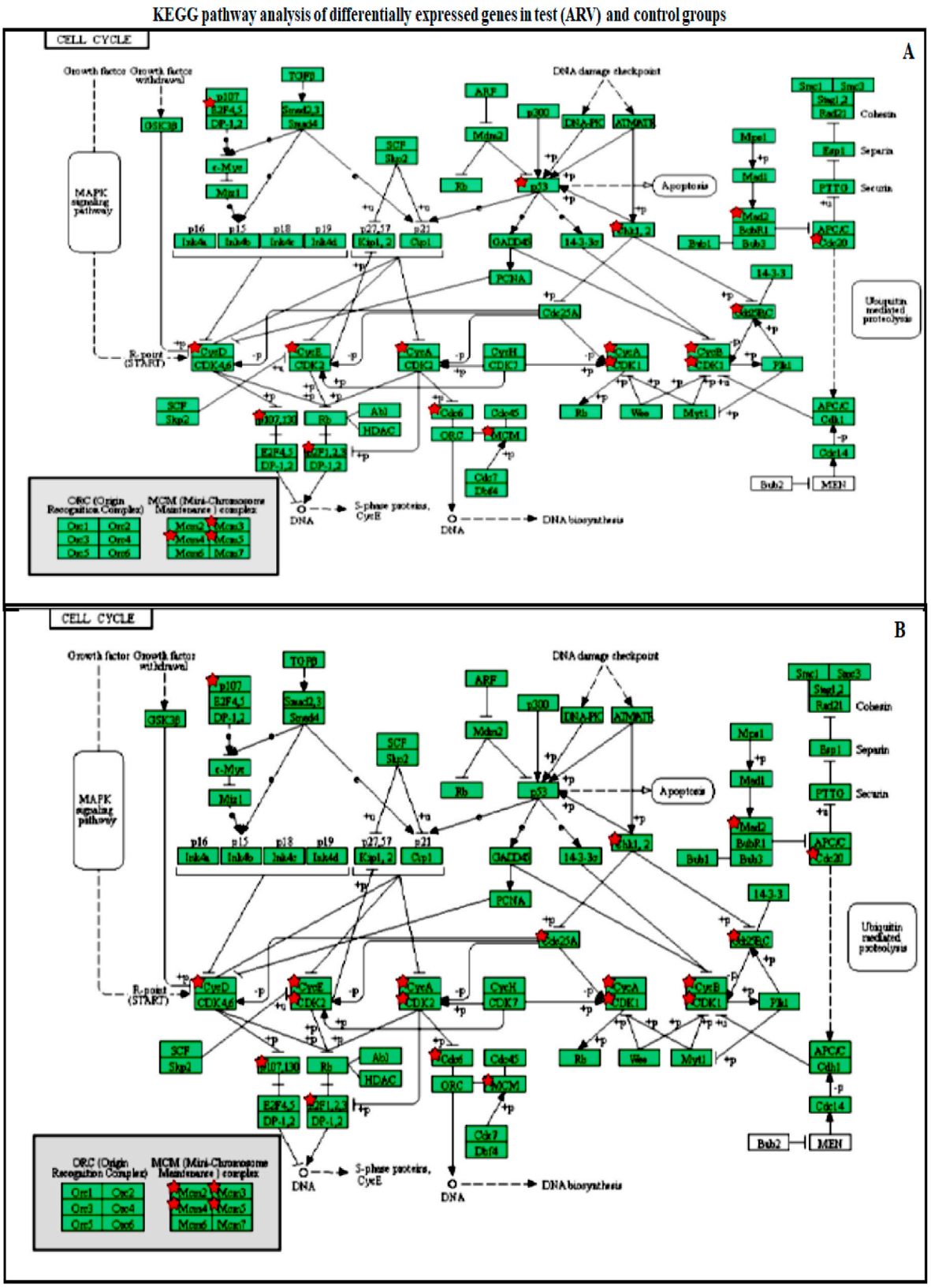

Fig. 4. KEGG pathway analysis of differentially expressed genes between ARV treated and control groups. **A** and **B** are a representation of upregulated genes prior to ARV exposure and downregulated gene targets in response to ARV treatment. **A** represents upregulated gene targets in the control group (A549 vs MRC-5), while **B** shows downregulated genes in response to ARV drug group. The EFV and the LPV/r treated groups show similar targeted response.

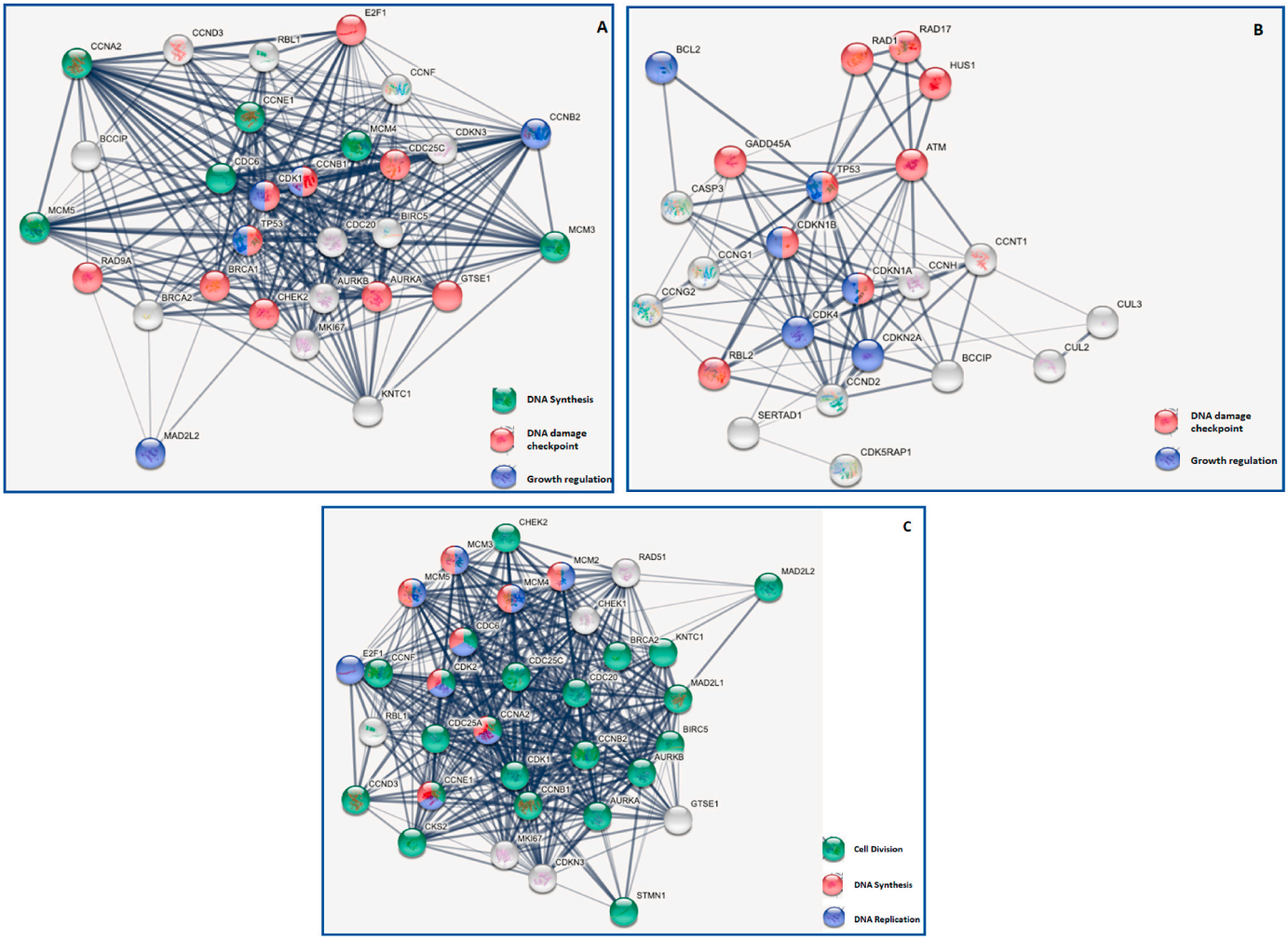

Fig. 5. STRING PPI network analysis of DEG targets. **A** demonstrates upregulated gene targets in control cells. **B** and **C** show up-and downregulated gene targets in ARV treated cells. Observations in EFV and LPV/r treated groups are similar.

cell cycle pathway (Fig. 4), while white boxes denote other pathways associated with the cell cycle. A representation of the results in ARV treated and non-ARV treated is shown here.

DAVID analysis identified the change in expression of cell cycle regulatory factors such as the cyclin/CDK activity, and the p53 down-stream targets. Even though the p53 tumour suppressor is not supressed in the cancer cells, its down-stream effector targets such as p21, GADD45A (to arrest growth and possibly repair the damaged DNA) are suppressed (Fig. 4A). In response to EFV exposure, ATM/ATR DNA damage checkpoints are activated, which in turn activates CHK1 and 2 in the normal cells; and also noteworthy is the upregulation of the CDK-inhibitor p15 (EFV-upregulated DEGs in MRC-5). On the other hand, p27/57 is upregulated by the EFV treatment in the A549 cells (EFV-upregulated DEGs in A549). Unlike in the control cells with repressed CDKI activity (Control-downregulated DEGs), the EFV treatment upregulates the CDK-inhibitors in both cancerous and normal cells, even though the cyclin (D and H)/CDK are active in normal cells. The activation of the CDKIs could be attributed to the drugs repressing progression of the cell cycle driven by the cyclin/CDK interactions. Similar to EFV, LPV/r treatment upregulates the CDKI-p15 which inhibits the activity of CDK/cyclin complexes, thereby inhibiting the progression of the cell cycle in normal cells (LPV/r-upregulated DEGs in MRC-5), Fig. 4B. Similarly, the activation of p16, p21 and p27/57 is observed in A549 LPV/r treated cells, leading to the reduced activity of the cyclin/CDK complexes in both normal and cancerous cells (LPV/r-down-regulated in both MRC-5 and A549). Both EFV and LPV/r target the activation of growth arrest genes, leading to the reduction in cell-cycle progression.

3.4. STRING analysis

STRING protein-protein interaction (PPI) analysis of the cell cycle genes evaluated here in response to ARV treatment elucidated a series of tight interactions. Pivotal factors were identified in relation to the DNA damage response and growth arrest including GADD45A, HUS and RAD gene products and are illustrated (Fig. 5) and discussed further below.

The regulatory role of p53 on BRCA1 activity is observed by the strong and direct interaction between these two molecules, while MAD2L2 is distant from the network. The elevated expression of DNA synthesizing genes is also shown here, Fig. 5A. The deregulation of CASP3 and RAD genes/proteins and HUS1 suggests the malfunctioning of the DNA damage sensors, and the non-functioning of the effector CASP3 in the lung adenocarcinoma cells, thereby preventing them from undergoing CASP3 mediated apoptosis. Additionally, negative regulators of the cell cycle, p15 and p21 are shown to be downregulated in this network. The EFV drug treatment upregulates the DNA damage response genes in both normal and cancerous cells, while repressing the expression of AURKB and MAD2L2 and cyclin B2-cell division genes in the A549 cells. In a similar manner, LPV/r drug exposure stimulates the expression of p53 and its downstream targets such as GADD45A (DNA damage response gene) in the A549 cells, while suppressing DNA replication genes (MCM), the cell division cycle genes CDC20 and

CDC25. In normal cells, most of the genes maintain a suppressed state, even post LPV/r exposure. Fig. 5B and C are a representation of the up and down regulated genes in response to ARVs. Overall, the ARV drugs stimulated DNA damage response pathways and repressed cell proliferation in both normal and cancer cells.

4. Discussion

Activated DNA damage sensors and signallers interact subsequent to ARV treatment, while these mechanisms remain inactivated in control groups. Furthermore, EFV and LPV/r drug treatment altered the expression of the profiled cell cycle related genes, affecting other pathways besides the cell cycle. In particular, EFV was shown to activate the cellular response to external stimuli genes in MRC-5 cells, DNA repair genes in both normal and cancerous cells, while upregulated PCD genes and an obvious decline in expression of genes involved in DNA replication in A549 cells was observed compared to the control cells. LPV/r treatment results in the reduced expression of DNA replication in both normal and cancerous cells, while an increase in genes involved in PCD in the cancerous cells was observed. Reducing/inhibiting DNA synthesis is an important therapeutic strategy employed by anti-cancer drugs [23]. These findings suggest a regulatory role of EFV and LPV/r on the progression of the cell cycle in lung cells. Additionally, FOXO transcription factors are critical for the regulation of cell cycle arrest, cell death, and DNA damage repair. Target genes that mediate FOXO induced cell cycle arrest include the CDK inhibitors p27 and p21 and cyclin D [32]. Even though p53 and FOXO mediated transcription share similar downstream targets, GO analysis reveals the activation of FOXO transcription factors. Furthermore, p27 or p21 targeted cyclin D G1 events tends to prolong the duration of the G1 phase, thereby delaying the S-phase entry and thus DNA replication and cell division [33,34]. Although currently these ARV drugs are used in the treatment of HIV/AIDS, their role in the regulation of the cell cycle, inducing the cycle arrest and activating the FOXO transcription factors and DDR, with the goal to reduce cell proliferation is evident here, particularly in the A549 adenocarcinoma cells. The anti-proliferative and cytotoxic effects of EFV and LPV/r on different cancer cells such as colorectal, pancreatic and cervical cancer have been previously reported [35,36].

Although there may be further steps to be considered, EFV and LPV/r demonstrate unique but also at the same time similar mechanisms, whereby they both target similar genes/products affecting cell cycle regulation. The recruitment of DNA damage response pathways following EFV and LPV/r treatment in both normal and cancer cells indicates that these drugs possess anti-tumour/anti-proliferative activity, similar to those demonstrated by chemotherapeutic drugs. In the same manner, people living with HIV/AIDS (PLWHA) previously not pre-disposed to cancer, particularly lung cancer, may be undergoing similar processes, where the administration of EFV and LPV/r impose damage to the genome, resulting in the activation of cellular response to external stimuli and DNA damage response genes/pathways. Being actively induced by consistent and continuous HAART administration, these pathways may be dysregulated (possibly due to constant activation), ultimately leading to the malfunctioning of the cell cycle checkpoints and then tumorigenesis [37,38]. The anti-proliferative and cytotoxic effects of ARV drugs is an emerging subject. Nelfinavir (PI), for example is in clinical trials as a radiosensitiser in the treatment of NSCLC [39]. However, in normal primary cells, the constant activation of DDR may eventually exhaust the cells' repair mechanisms, bypassing cell cycle checkpoints and leading to uncontrolled cell proliferation and tumorigenesis [40,41]. This however, does not exclude ARVs from being explored as anti-cancer drugs, due to their anti-cancer properties.

5. Limitations

Even though the doses used in this study reflect the clinical relevant doses, and provide a good indication of the events following ARVs intake, the duration of cells exposure to these drugs is minimal, as HIV/AIDS patients on HAART are exposed to these drugs over a longer period. It would also be interesting to study these ARVs effects on lung cells in the presence on the HI-provirus. As A549 represents the adenocarcinoma, accounting for many lung cancer cases, the role of these ARVs in other lung cancer models, or other types of cancer would be interesting to study.

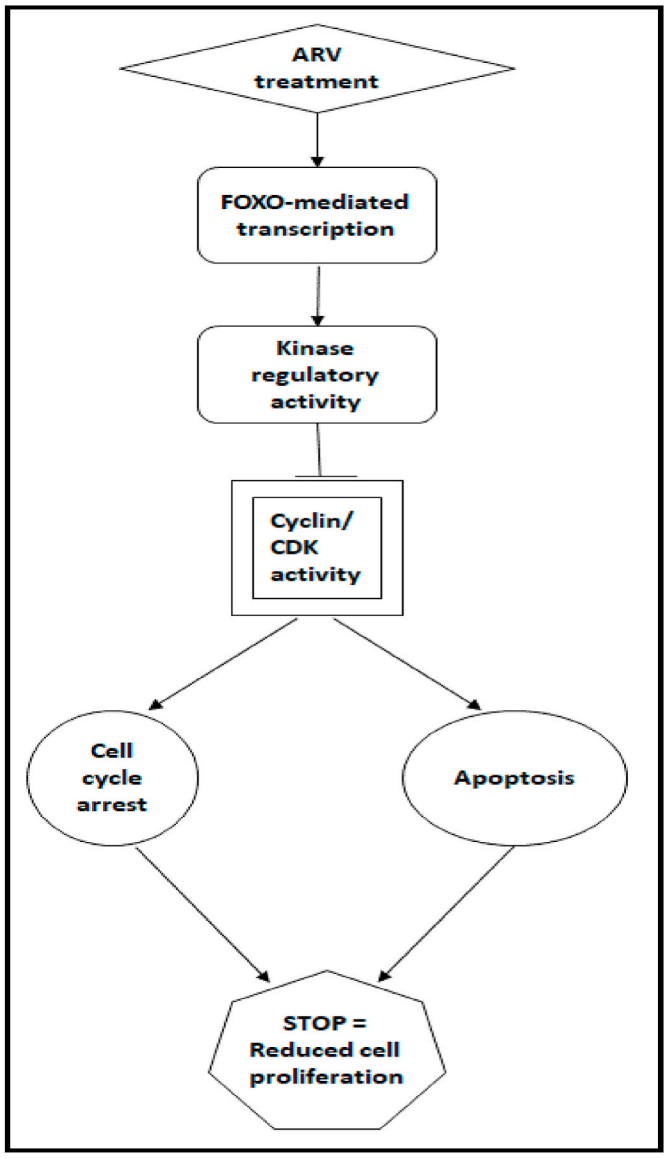

Fig. 6. The summarised mode of mechanism of ARVs in lung cell regulation. The FOXO-mediated transcription factors targeting the kinase regulatory activity such as p27 and p21 halts the cell cycle progression by targeted protein/kinase/cyclin binding. Depending on the motive of the cell cycle arrest, cell death may occur. This ARV's FOXO mediated transcription reduces cell proliferation, a characteristic of anti-cancer drug. This similar mechanism may also be observed in p53 cell cycle regulation.

6. Conclusions

The targeted mode of cell cycle regulation by these ARVs is summarised in Fig. 6. It is clear that these ARVs have a regulatory role on the cell cycle, by targeting key regulators of the cell-cycle. The ultimate downstream effect of these drugs is to slow down cell proliferation by arresting the cell cycle or inducing cell death.

Author contributions

Rahaba Marima and Clement Penny conceived and designed the study. Rahaba Marima performed the experiments. Rahaba Marima and Jeyalakshmi Kandhavelu analysed the data. Rahaba Marima, Rodney Hull, Jeyalakshmi Kandhavelu, Zodwa Dlamini and Clement Penny drafted the manuscript. Jeyalakshmi Kandhavelu, Zodwa Dlamini and Clement Penny reviewed and edited the manuscript. All authors read and approved the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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